ACTA OBST GYNAEC JPN Vol. 37, No. 12, pp. 2847—2852, Dec. 1985

ALTERATIONS IN TIGHT JUNCTIONS OF HUMAN ENDOMETRIAL EPITHELIAL CELLS DURING NORMAL MENSTRUAL CYCLE —FREEZE-FRACTURE ELECTRON MICROSCOPIC STUDY—

Shigeaki IWANAGA*,**, Tetsuo INOKUCHI**, Akitoshi NOTOHARA**, Ryuhei HIGASHI***, Masahiro MURAKAMI** and Toshi KATO*

Department of Obstetrics and Gynecology, Kurume University School of Medicine, Kurume

***Department of Anatomy, Kurume University School of Medicine, Kurume*

****Laboratory of Electron Microscopy, Kurume University School of Medicine, Kurume*

Synopsis The structural change in the tight junction between human endometrial epithelial cells during the normal menstrual cycle was observed by means of freeze-fracture replica electron microscopy.

Tight junctions between epithelial cells of the endometrial basal layer showed no alterations in structure during the menstrual cycle, while those between epithelial cells of the functional layer exhibited remarkable changes synchronized with the menstrual cycle; in the early proliferative phase, the tight junctions were composed of strands running mainly parallel to the luminal surface and the depth of the junctions from the luminal surface was $0.57\pm0.04\mu$m (Mean ±S.E.), wheras in the late proliferative phase the junction formed a prominent network of strands $0.80\pm0.04\mu$m deep. In the early secretory phase, the junctions developed mostly as a complex network, and the depth was $0.96\pm0.04\mu$m. In the late secretory phase, the network consisting of the junctions looked disorderly and the depth of the junctions was reduced to $0.71\pm0.04\mu$m.

As a conclusion, it was suggested that the tight junction in the human endometrial epithelium tended to develop or diminish according to the phase of the menstrual cycle.

Key words: Tight junctions • Human endometrium • Epithelial cells

Introduction

It is well-known that the ultrastructure of the human endometrial epithelial cells shows marked changes during the menstrual cycle[3)11)27)~29)]. In detail, few reports have been made on the structural changes of the tight junction between these epithelial cells[6)12)16)].

Previous experiments using the freeze-fracture replica method revealed that the structure of the tight junctions was altered following the direct influence of the ovarian hormones in rats and rabbits[15)26)]. Murphy et al. (1982)[16)] observed the human endometrium with the freeze-fracture method and reported that the structure of tight junctions between the epithelial cells was changed according to the menstrual cycle. However, this report was limited only to the observation in the middle (day 14-16) and later (day 25-26) stages of the menstrual cycle and hence failed to make detailed observations during the whole menstrual cycle.

The authors intended to clarify the relationships between the human menstrual cycle and the structural changes of tight junctions in the endometrial epithelial cells. The junctions in the epithelial cells of basal and functional layers of the endometrium were comparatively examined in all the stages (early and late proliferative, early and late secretory phases) of the menstrual cycle.

Materials and Methods

Thirteen endometria in various stages of the menstrual cycle were taken from the human uteri removed surgically for various gynecologic indications; 3 in the early proliferative phase, 3 in the late proliferative phase, 4 in the early secretory phase and 3 in the late secretory phase. Each extripated specimen was divided to two pieces. Any material from abnormal endometria was excluded. Endometrial tissue with subjacent myometrium was obtained from anterior or posterior uterine wall.

One piece of each specimen was prepared for light microscopy to decide the menstrual phase of the endometrium according to the criteria of Noyes et al.[18].

Another piece was fixed for freeze-fracture replica study in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer. Under a stereo microscope the tissue piece was cut into such two portions as the functional layer including the surface epithelium and the basal layer adjacent to the myometrium. After the fixation the tissue specimen was frozen in Freon 22, transfered immediately to liquid nitrogen and fractured in a freeze-etching apparatus (Eiko FD-2A) at a temperture of −120℃. Replicas of the fracture face were made by evaporating platinum and carbon, and examined with a transmission electron microscope (Hitachi H-500).

Results

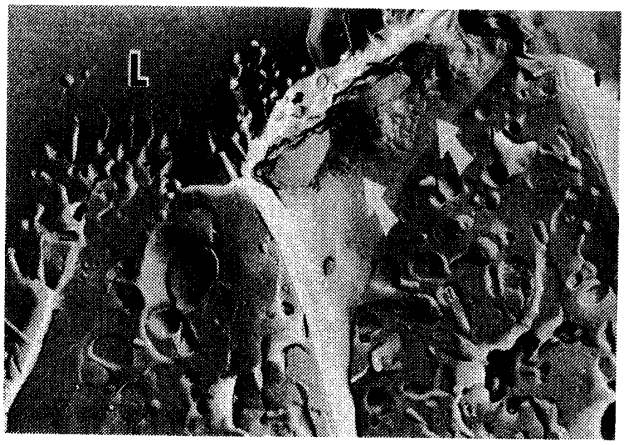
Prominent tight junctions were found at the luminal edges of the lateral cell membranes in the human endometrial epithelial cells. The junctions appeared as branching and anastomosing ridges of strands of particles on the P-face and as complementary furrows on the E-face (Fig. 1).

The human endometrial epithelium is composed of two types of cells, ciliated and non-ciliated. It has been reported that there is a structural difference of tight junctions between ciliated and non-ciliated cells in the mouse oviduct epithelium[13)25)]. In the present author's observations on the human endometrial epithelium no structural difference of the junctions was recognized between these two types of cells.

1. Tight junctions in the epithelium of the functional layer

During the menstrual cycle the junctions in the epithelium of the functional layer indicated distinct structural alterations as shown in Table 1 and Fig. 2-5.

Fig. 1. Freeze-fracture replica image of the human endometrial epithelial cells. A tight junction is seen at the luminal edge of the lateral cell membrane (arrow). L: lumen. ×14,000



The junctions in the early proliferative phase were composed of 8.0±1.0 (Mean ±S.E.) strands, among which several strands on the luminal side sometimes showed ramification or anastomosis, while those on the abluminal side revealed a pattern running in parallel with the luminal surface accompanying with few branches or anastomoses (Fig. 2). The depth of the junctions was 0.57±0.04μm (Mean ±S.E.) from the luminal surface.

The junctions in the late proliferative phase were made up of 9.4±0.9 strands, and the junctional pattern showed more complex branching or anastomosing than those in the early proliferative phase with forming a network (Fig. 3). The depth of the junctions of 0.80±0.04μm in the late proliferative phase was significantly deeper ($p<0.01$) than that in the early proliferative phase.

The junctions in the early secretory phase had

Table 1. Measurement of the tight junctions in the endometrial functional layer.

	early proliferative phase	late proliferative phase	early secretory phase	late secretory phase
Depth of junction (μm)				
Range	0.34−0.74	0.65−1.00	0.74−1.04	0.50−0.88
Mean±SE	0.57±0.04	0.80±0.04	0.96±0.04	0.71±0.04
Number of strands				
Range	6 −11	6 −11	9 −12	8 −12
Mean±SE	8.0±1.0	9.4±0.9	10.8±0.6	10.4±0.7

Measurements were made parallel to the luminal surface.

Fig. 2. The tight junction of the functional layer in the early proliferative phase of the endometrium. The junctional strands run more or less parallel to the luminal surface. LS : luminal surface. ×37,000

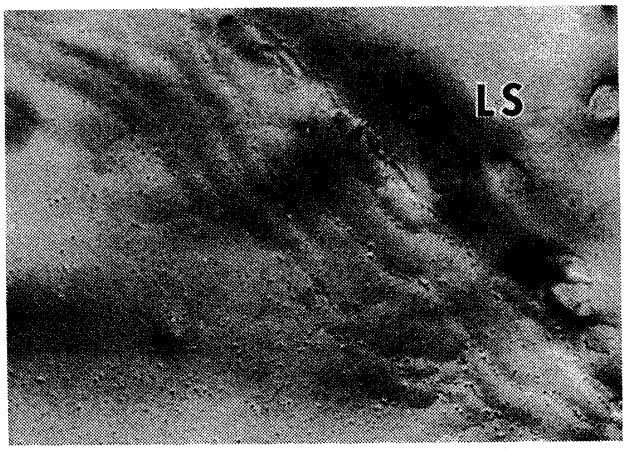
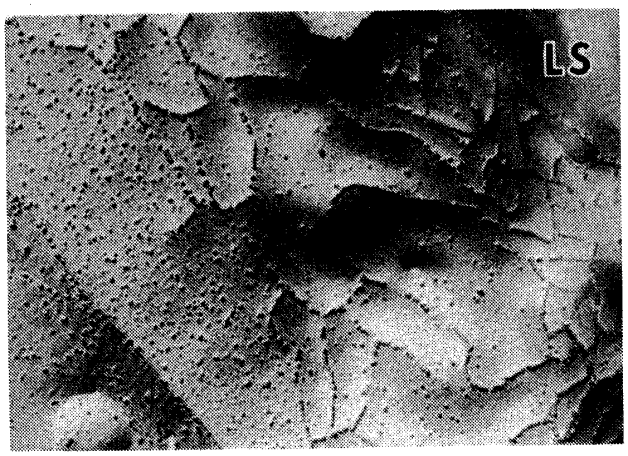


Fig. 3. The tight junction of the functional layer in the late proliferative phase of the endometrium. Strands making the tight junction show network profile. LS : luminal surface. ×52,000



10.8 ± 0.6 strands, and the junctional pattern showed a more complex network of anastomosing strands, comparing to those in the late proliferative phase (Fig. 4). The depth of the junctions in this phase was deeper than that in the early and late proliferative phase, indicating $0.96 \pm 0.04 \mu$m.

The junctions in the late secretory phase were composed of 10.4 ± 0.7 strands which arranged disorderly to form the network. The depth of the junctions was $0.71 \pm 0.04 \mu$m and significantly shallower ($p < 0.01$) than that in the early secretory phase (Fig. 5).

Fig. 4. The tight junction of the fufnctional layer in the early secretory phase of the endometrium. The junction shows complex network. LS : luminal surface. ×38,000

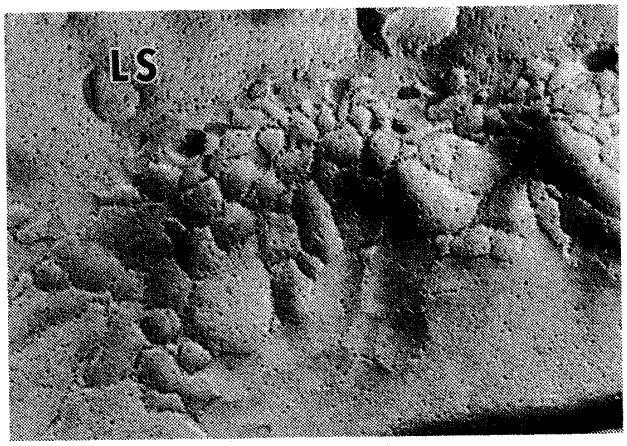


Fig. 5. The tight junction of the functional layer in the late secretory phase of the endometrium. Strands making the tight junction show irregularly arranged network. LS : luminal surface. ×27,000

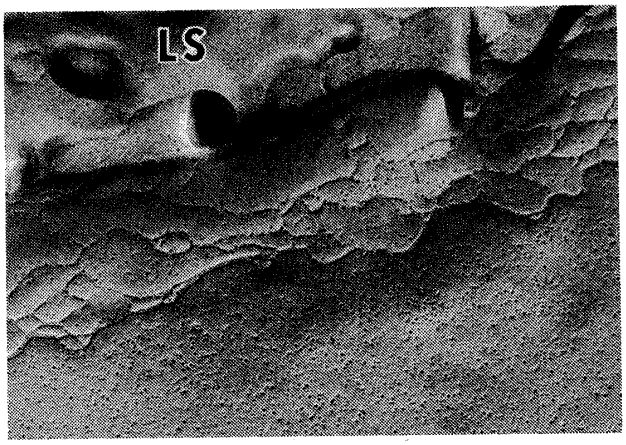


Fig. 6. The tight junction in the basal layer of the endometriun. Throughout the menstrual cycle the junctional pattern is similar to that shown in Fig. 4. L : lumen. ×26,000

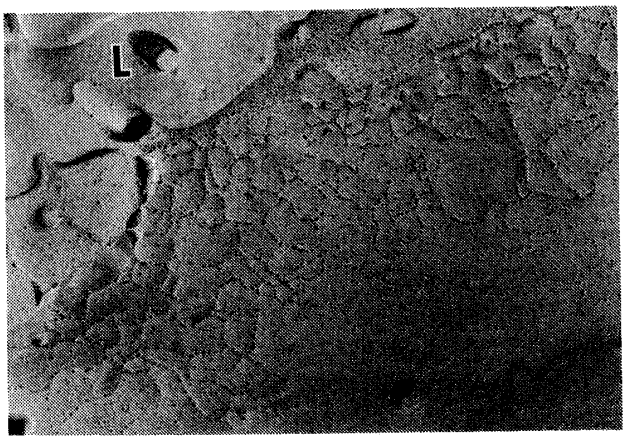
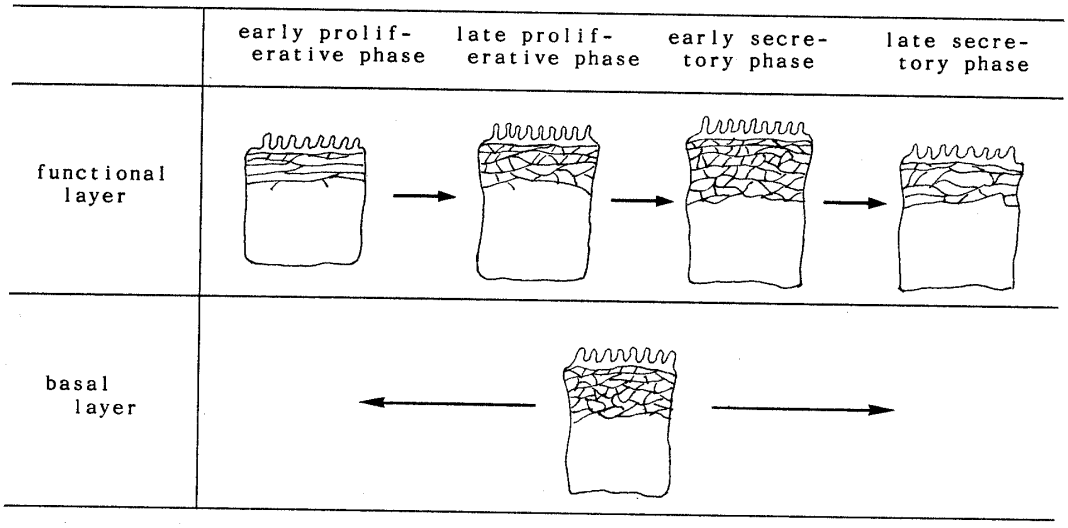


Fig. 7. Schematic representation of the tight junctions in human endometrial epithelium.
The tight junctions in the epithelium of the endometrial basal layer show no remarkable alterations in structure during the menstrual cycle, while those of the epithelium of the functional layer exhibit marked changes.



2. Tight junctions in the epithelium of the basal layer

The tight junctions in the epithelium of the basal layer did not show marked changes during the menstrual cycle. Throughout the cycle, a complex network was recognized and was similar to that of the junctions as noted in the epithelium of the functional layer in the early secretory phase (Fig. 6). Principal results of this study are summarized in Fig. 7.

Discussion

It has been described that the size and distribution of the tight junctions in various epithelia can be change by the functional state of the epithelium or following after the influence of hormones or pharmacological agents[14)15)19)~21)23)24)]. It has been also reported that, in rats[15)] and rabbits[26)], the tight junctions in the endometrial epithelial cells show marked structural changes by administration of ovarian hormones. According to previous studies, the junctional ridges run mainly in parallel with the luminal surface under the influence of estrogen, while progesteron makes the junctions more developed and complex.

In the present study on the human endometrial epithelial cells, the junctions of the functional layer in the proliferative phase, during which no effect of progesteron was suggested, became larger and showed more complex network than that in the early proliferative phase. This was a contradictory finding to those in rats and rabbits. Unlike the case in rats and rabbits, the tight junctions in human endometrial epithelium showed rather structural degradation in the late secretory phase as compared with that in the early secretory phase (Table 1 and Fig. 7). This finding agrees with the report by Murphy et al. (1982)[16)] who stated that the tight junctions in human endometrium were deeper and more extensive in the middle stage of the menstrual cycle than they were in the later.

Unlike the endometrium in rats and rabbits, the functional layer of the human endometrium desquamates transiently during menstruation and thereafter regenerates, proliferates and develops from the residue[5)9)10)17)27)]. The fashion of repetition of development of the epithelium in the functional layer of the human endometrium resembles the development of the epithelium in the intestinal mucosa[2)].

Polak-Charcon et al. (1980)[20)], who observed Lieberkuhn's crypts in human colonic mucosa with freeze-fracture method, reported that the tight junctions between the epithelial cells soon after their division at the base of the crypts formed more complex network and greater depth as the cells matured and moved upwards. The similar finding was reported in rat intestinal crypt cells by Tice et

al. (1979)[23].

As in intestinal crypt cells, the tight junctions of the epithelium in the functional layer of the human endometrium may secondarily develop in accordance with development of the whole epithelium during the menstrual cycle. The disassembly of the strands of the junctions of the epithelium in the functional layer of human endometrium in the late secretory phase may be regarded as the same as a phenomenon that the epithelial cells regress in the late secretory phase after a fall in ovarian hormone levels in the late secretory phase[3)5)11)27)].

The present observation has clarified that the tight junctions of the epithelium in the basal layer did not demonstrate marked structural change throughout the period of the menstrual cycle, and this fact is in good accord with the previous finding that no change in epithelium of the basal layer can be seen during the menstrual cycle[1)17)].

The tight junction between the epithelial cells is generally considered to separate the lumen from the surrounding tissue and permit the epithelium to create biochemical gradients between their lumina and underlying tissue[4)7)8)22)]. The tight junctions in the endometrial epithelial cells may have a role in controlling the volume and the composition of the uterine luminal fluid, and possibly they influence directly humoral emvironment of the embryo[15)26)]. It is pointed out, however, that the practical functions of the junctions in the human endometrial epithelium must be further clarified in future studies.

References

1. *Blandau, R.J.:* The female reproductive system. In Historogy, 4th eds. (eds. L. Weiss and R.O. Greep), 881. McGraw-Hill, Inc., New York, 1977.
2. *Bloom, W. and Fawcett, D.W.:* In A Textbook of Histology, 10th ed., W.B. Saunders Co., Philadelphia, London, 1975.
3. *Cavazos, F., Green, J.A., Hall, D.G. and Lucas, F. V.:* Ultrastructure of the human endometrial glandular cell during the menstrual cycle. Am. J. Obstet. Gynecol., 99 : 833, 1967.
4. *Claude, P. and Goodenough, D.A.:* Fracture faces of zonulae occludentes from "tight" and "leaky" epithelia. J. Cell Biol., 58 : 390, 1973.
5. *Dallenbach-Hellwich, G.:* In Histopathology of the Endometrium, 3rd ed., 22. Springer-Verlag, Berlin, Heidelberg, 1981.
6. *Davie, R., Hopwood, D. and Levison, D.A.:* Intercellular spaces and cell junctions in endometrial glands: Their possible role in menstration. Br. J. Obstet. Gynecol., 84 : 467, 1977.
7. *Farquhar, M.G. and Palade, G.E.:* Junctional complexes in various epithelia. J. Cell Biol., 17 : 375, 1963.
8. *Fawcett, D.W.:* Junctional specializations. In The Cell, 2nd ed., 124. W.B. Saunders Company, Philadelphia, 1981.
9. *Ferenczy, A.:* Studies on the cytodynamics of human endometrial regeneration. I. Scanning electron microscopy. Am. J. Obstet. Gynecol., 124 : 64, 1976.
10. *Ferenczy, A.:* Studies on the cytodynamics of human endometrial regeneration. II. Transmission electron microscopy and histochemistry. Am. J. Obstet. Gynecol., 124 : 582, 1976.
11. *Gordon, M.:* Cyclic changes in the fine structure of the epithelial cells of human endometrium. Int. Rev. Cytol., 42 : 127, 1975.
12. *Heinrich, D.:* Zur Morphologie der Zellverbindungen des menschelichen Endometriums. Verh. Anat. Ger., 72 : 235, 1978.
13. *Komatsu, M., Ishimura, K. and Fujita, H.:* Freeze-fracture images of the zonula occludens in the mouse oviduct epithelium. Arch. Histol. Jap., 41 : 453, 1978.
14. *Montesano, R., Gabbiani, G., Perrelet, A. and Orci, L.:* In vivo induction of tight junction proliferation in rat liver. J. Cell Biol., 68 : 793, 1976.
15. *Murphy, C.R., Swift, J.G., Mukherjee, T.M. and Rogers, A.W.:* Effects of ovarian hormones on cell membranes in the rat uterus. II. Freeze-fracture studies on tight junctions of the lateral plasma membrane of the luminal epithelium. CELL BIOPHYSICS, 3 : 57, 1981.
16. *Murphy, C.R., Swift, J.G., Need, J.A., Mukherjee, T. M. and Rogers, A.W.:* A freeze-fracture electron microscopic study of tight junctions of epithelial cells in the human uterus. Anat. Embryol., 167 : 367, 1982.
17. *Novac, E.R. and Woodruff, J.D.:* Histology of the endometrium. In Gynecologic and Obstetric Pathology, 10th ed., 171. W.B. Saunders Co., Philadelphia, 1979.
18. *Noyes, R.M., Hertig, A.T. and Rok, J.:* Dating of the endometrial biopsy. Fertil. Steril., 1 : 3, 1950.
19. *Pitelka, D.R., Hamamoto, S.T., Duafara, J.G. and Nemanic, M.K.:* Cell contacts in the mouse mammary gland. 1. Normal gland in postnatal development and the secretory cycle. J. Cell Biol., 56 : 797, 1973.
20. *Polak-Charcon, S., Shoham, J. and Ben-Shaul, Y.:* Tight junctions in epithelial cells of human fetal hindgut, normal colon, and colon adenocarcinoma.

JNCL, 65 : 53, 1980.
21. *Robenek, H., Themann, H. and Ott, K.:* Carbon tetrachloride induced proliferation of tight junctions in the rat liver as revealed by freeze-fracturing. Eur. J. Cell Biol., 20 : 62, 1979.
22. *Staehelin, L.A.:* Structure and function of intercellular junctions. Int. Rev. of Cytol., 39 : 191, 1974.
23. *Tice, L.W., Carter, R.L. and Cahill, M.B.:* Changes in tight junctions in rat intestinal crypt cells associsted with changes in their mitotic activity. Tissue Cell, 11 : 293, 1979.
24. *Tice, L.W., Wollman, S.H. and Carter, R.C.:* Changes in tight junctions of thyroid epithelium with changes in thyroid activity. J. Cell Biol., 66 : 657, 1975.
25. *Toshimori, K., Higashi, R. and Oura, C.:* Quantitive analysis of zonulae occludentes between oviductal epithelial cells at diestrous stages in the muose : Freeze-fracture study. Anat. Rec., 206 : 257, 1983.
26. *Winterhager, E. and Kühnel, W.:* Alterations in intercellular junctions of the uterine epithelium during the preimplantation phase in the rabbit. Cell Tissue Res., 224 : 517, 1982.
27. *Wynn, R.M.:* Histology and ultrastructure of the human endometrium. In Biology of the uterus, (ed. R.M. Wynn), 341. Plenum Press, New York, 1977.
28. *Wynn, R.M. and Harris, J.A.:* Ultrastructural cyclic changes in the human endometrium. 1. Normal preovulatory phase. Fertil. Steril., 18 : 632, 1967.
29. *Wynn, R.M. and Wooley, R.S.:* Ultrastructural cyclic changes in the human endometrium. 2. Normal postovulatory phase. Fertil. Steril., 18 : 721, 1967.

(Accepted : No. 5840, September 26, 1985)

概要 正常月経周期における，ヒト子宮内膜上皮細胞間に存在する閉鎖帯（tight junction）を freeze-fracture replica 法を用いて電子顕微鏡的に観察した．

子宮内膜基底層の上皮に存在する閉鎖帯は，月経周期を通じてその構造に著明な変化を示さなかつた．しかしながら，機能層の上皮に存在する閉鎖帯は，月経周期に伴つて上皮細胞の外観が示す形態の変化と同調した構造の変化を示した：増殖期初期の閉鎖帯は主に内腔表面に平行に走る strands で構成され，その内腔面からの深さは0.57±0.12μm（Mean ±S.E.）であつた．増殖期後期には閉鎖帯の strands は全体として網目状の構造をとり，深さは0.79±0.12μm であつた．分泌期初期には閉鎖帯は最も発達しており，密な網目状構造を示し，深さは0.99±0.1μm となつた．分泌期後期には閉鎖帯の strands が作る網目状構造は乱れを示し，その深さも浅くなり0.71±0.04μm となつた．

結論として，ヒト子宮内膜上皮細胞間に存在する閉鎖帯は，卵巣ホルモンの影響を直接うけて閉鎖帯の構造に変化を示すとされるラットやウサギ子宮の場合と異なり，月経周期に伴う上皮の形態変動に従つて二次的に発達及び退行するものと考えられる．